T1 MATRIX METALLOPROTEINASE-DRIVEN TISSUE DESTRUCTION IN HUMAN TUBERCULOSIS (TB) IS MEDIATED BY TH-17 CYTOKINES AND THE PI3K/P110A/P70S6K CASCADE

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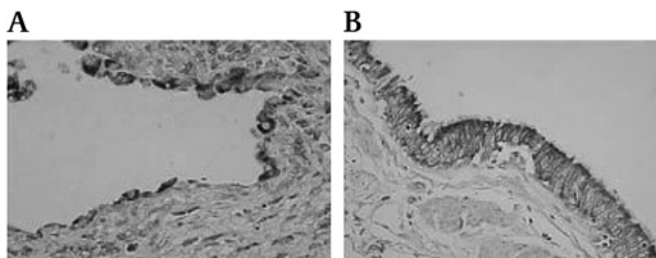
[1]S Singh, [2]U K Singh, [1]P T Elkington, [1]J S Friedland. [1]*Imperial College London, London, UK;* [2]*Nalanda Medical College and Hospitals, Patna, India*

Mycobacterium tuberculosis (Mtb) kills 1.7 million people annually. The Th1 paradigm does not explain TB-driven cavitation. Current treatment is lengthy with many adverse effects. The Interleukin-23/Th17 axis plays a critical role in early Mtb containment. Respiratory stromal cells are important first-line defence and secrete Matrix metalloproteinases (MMPs). Role of MMPs, which are substrate-specific proteases causing extracellular matrix degradation/remodelling, was investigated in TB. Human bronchoalveolar lavage (BAL) samples from 35 well-characterised TB and control patients were analysed for MMPs and Th17 cytokines. TB/Control lung biopsies were stained for MMPs/IL-17. Primary normal human bronchial epithelial cells (NHBEs) and MRC-5 fibroblasts were stimulated with IL-17/IL-22/IL-23, alone and in combination with conditioned medium from Mtb-infected monocytes (CoMTb). Secretion, gene expression, gene silencing, intracellular signalling were investigated by luminex, ELISA, zymography, dual-luciferase promoter-reporter, realtime RT-PCR, siRNA transfection. MMPs were up-regulated in Human TB BALs ($p<0.0001$). This positively correlated with cavitation score on CXRs. TIMPs (tissue inhibitor of metalloproteinase) and IL-17/IL-23 were unaltered but IL-22 was increased in TB BAL. IL-17 and MMP-3 were co-expressed in pneumocytes around granulomas in TB lung biopsies. CoMTb (but not direct infection) up-regulated secretion and gene expression of MMP-1 (collagenase, $p<0.0001$), MMP-3 (stromelysin, $p<0.001$) and MMP-9 (gelatinase, $p<0.0001$) from NHBEs. MMP-3 protein and promoter activity in MRC-5s was also increased ($p<0.001$). AKT inhibition suppressed all MMPs ($p<0.01$) whereas siRNA and chemical inhibition of the proximal PI3Kp110a subunit abrogated MMP-3 only ($p<0.001$). Distally, p70S6K (mTOR) blockade with rapamycin abrogated TB-driven MMP-1 and MMP-3 ($p<0.001$). MMP-9 production was unaffected by proximal/distal inhibition of PI3K.IL-17 independently and also synergistically with CoMTb augmented MMP-3 secretion/gene expression from NHBEs and MRC-5s in a dose-dependent manner (peak 8ng/ml, $p<0.0001$). This was p38-dependent, confirmed by p38-specific siRNA. In contrast, IL-17 down-regulated CoMTb-driven MMP-9 to baseline ($p<0.01$). IL-22 augmented MMP-3 from fibroblasts but not from NHBEs. IL-23 did not drive MMPs.

Summary MMPs are key mediators of tissue damage in human pulmonary TB and are regulated in a cell- and stimulus-specific manner. IL-17 and IL-22 drive MMP-3 but suppress MMP-9 in airway epithelium. The PI3Kinase/p110a/p70S6K pathway is a crucial target and its immuno-modulation (eg, rapamycin) is a potential adjunctive therapy to limit tissue destruction and shorten chemotherapy in TB.



Abstract T1 Figure 1 Pulmonary epithelial cells around a TB granuloma expressed IL-17 (A). Strong immunoreactivity for MMP-3 (B) in pulmonary epithelial cells around TB granulomas was also detected. Control lungs (not shown) were negative.

T2 SINGLE NUCLEOTIDE POLYMORPHISMS IN THE FICOLIN-2 GENE PREDISPOSE TO *PSEUDOMONAS AERUGINOSA* INFECTION AND DISEASE SEVERITY IN NON-CYSTIC FIBROSIS BRONCHIECTASIS

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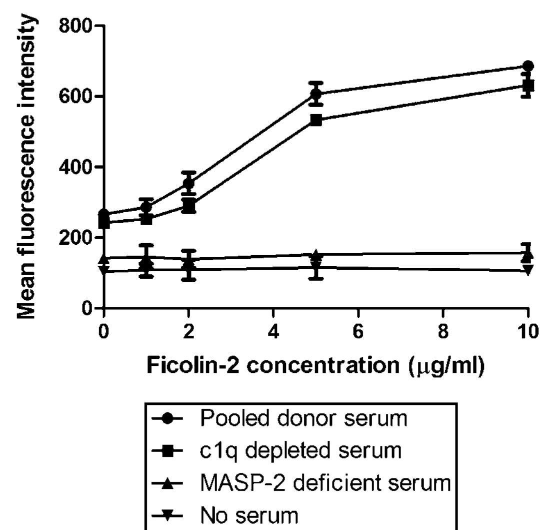
[1]J D Chalmers, [2]D C Kilpatrick, [1]B J McHugh, [1]M P Smith, [1]J R W Govan, [1]C Doherty, [3]M Matsushita, [4]S P Hart, [5]T Sethi, [1]A T Hill. [1]*University of Edinburgh, Edinburgh, UK;* [2]*Scottish National Blood Transfusion Service, Edinburgh, UK;* [3]*Tokai University, Kanagawa, UK;* [4]*University of Hull, Hull, UK;* [5]*King College, London, UK*

Introduction Bronchiectasis is associated with a destructive cycle of bacterial infection, airway inflammation and airway structural damage. Ficolins are a family of recently described serum pattern recognition molecules capable of binding to micro-organisms and activating complement through MBL associated serine protease-2 (MASP-2). Their role in chronic lung disease has not previously been investigated.

Methods Serum levels of Ficolin-2 and MASP-2 were determined in 470 patients with idiopathic non-cystic fibrosis bronchiectasis and 400 matched control subjects by ELISA. Single nucleotide polymorphisms were determined using TaqMan PCR based genotyping. Bacterial binding was determined using ELISA and flow cytometry. Neutrophils from healthy donors were isolated by percoll gradient centrifugation and used in phagocytosis assays with FITC-labelled *Pseudomonas aeruginosa* strain PA01.

Results Genotyping success was >95% and all SNP's were in Hardy–Weinburg equilibrium ($p>0.05$). Bronchiectasis was associated with a homozygous mutation in the promoter of ficolin-2 (rs3124952) (28.5% vs 19.5%, $p=0.002$) and a homozygous mutation in exon-8 of the ficolin-2 gene causing impaired binding (rs17549193) (12% vs 8.4%, $p=0.04$). Low serum levels (<1.6 μg/ml, 2 SD. below the mean for controls) were strongly associated with the promoter polymorphisms ($p<0.0001$) and with bronchiectasis (18.7% vs 7.8%, $p<0.0001$). In-vitro, ficolin-2 bound to over 60 strains of *P aeruginosa*, including clinical isolates and mucoid strains, activating the lectin pathway of complement and promoting C3 and C4 deposition. Ficolin-2 also bound to *Streptococcus pneumoniae,*



Abstract T2 Figure 1 Phagocytosis of FITC labelled *Pseudomonas aeruginosa* by neutrophils is enhanced by recombinant Ficolin-2 in the presence of complement.

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Haemophilus influenzae and *Staphylococcus aureus*. Opsonisation with ficolin-2 promoted phagocytosis of *P aeruginosa* (PA01) by human neutrophils in a MASP-2 but not c1q dependent manner ($p<0.0001$) (Abstract T2 figure 1). On multivariable analysis chronic bacterial colonisation (OR=3.5; $p<0.0001$) and particularly *P aeruginosa* colonisation (OR=2.8, p=0.0001) were independently associated with ficolin-2 insufficiency. These patients also had more frequent outpatient exacerbations (mean 3.2/yr vs 2.4/yr, p=0.01) and unscheduled hospital admissions for exacerbations (OR=2.3; $p<0.0001$).

Conclusion Single nucleotide polymorphisms in the ficolin-2 gene affecting serum levels and carbohydrate binding are associated with non-CF bronchiectasis and increase susceptibility to colonisation with *P aeruginosa*.

T3 MEASURING EOSINOPHIL KINETICS IN HUMANS

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[1]N Farahi, [1]A M Condliffe, [1]N R Singh, [2]S Heard, [1]R P Simmonds, [2]C K Solanki, [2]K Solanki, [2]K K Balan, [1]A M Peters, [1]E R Chilvers. [1]*University of Cambridge, Cambridge, UK;* [2]*Addenbrooke's Hospital, Cambridge, UK*

Introduction and Objectives Eosinophils are major cellular effectors of allergic inflammation and represent an important therapeutic target in asthma. While much is understood about the generation and activation of eosinophils, little is known about their intravascular kinetics and physiological fate. The purpose of this study was to image sites of eosinophil distribution and measure eosinophil kinetics in healthy individuals using autologous 111-Indium-labelled eosinophils.

Methods To determine "gold standard" kinetics of minimally-manipulated eosinophils, mixed leucocytes were isolated from the blood of healthy volunteers, labelled with 111-Indium-tropolonate and re-injected. Blood was sampled 0.75–72 h post-injection. Neutrophils and eosinophils were isolated in parallel, and cell-associated radioactivity was measured. To image sites of eosinophil margination/uptake eosinophils were purified using plasma-Percoll gradients and anti-CD16 immunomagnetic beads, labelled with 111-Indium-tropolonate and re-injected. The distribution of eosinophils was recorded by γ camera dynamic imaging (0–40 min) followed by static imaging up to 72 h.

Results Using minimally manipulated granulocytes we found that the 45min neutrophil recovery was 57±10% (n=7) and the intravascular lifespan was 10.3±0.1 h, in agreement with previous studies. By contrast, the 45min recovery of eosinophils was 15±2% (n=7) and eosinophil lifespan was 25.2±3.8 h. Moreover, eosinophils appeared to re-circulate at ~4 h and 9 h before mono-exponential removal. Using 111-Indium-eosinophils and γ camera imaging, we demonstrated initial retention of cells in the lungs, clearing to baseline by 40 min, with some early accumulation in the liver and progressive accumulation in the spleen (n=3). Simultaneous blood sampling showed that the 45 min recovery and intravascular lifespan of purified labelled eosinophils were 11±2% and 29.3±2.1 h, respectively, comparable to minimally manipulated cells. Purified cells also exhibited recirculation at ~6 h and 12 h. Of note, the disappearance of eosinophils from the liver at 6 h and 9 h coincided with their re-appearance in circulating blood, suggesting the liver as a possible site of transient eosinophil sequestration.

Conclusions This work provides the first in vivo measurements of eosinophil kinetics in healthy volunteers. Our data suggest that 111-In-labelled-eosinophils can be used to monitor the organ distribution and fate of eosinophils non-invasively. This technique may have an important role in assessing the therapeutic effects of eosinophil-targeted drugs.

T4 SAFETY AND EXPRESSION OF A SINGLE DOSE OF LIPID-MEDIATED CFTR GENE THERAPY TO THE UPPER AND LOWER AIRWAYS OF PATIENTS WITH CYSTIC FIBROSIS

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[1]G Davies, [1]J C Davies, [2]D R Gill, [2]S C Hyde, [3]C Boyd, [3]J A Innes, [3]D J Porteous, [4]S H Cheng, [4]R K Scheule, [5]T Higgins, [1]U Griesenbach, [5]E W F W Alton. [1]*Imperial College, London, UK;* [2]*University of Oxford, Oxford, UK;* [3]*University of Edinburgh, Edinburgh, UK;* [4]*Genzyme Corporation, Massachusetts, USA;* [5]*Fibrosis Gene Therapy Consortium, UK*

Introduction and Objectives We undertook a clinical trial of non-viral *CFTR* gene therapy assessing safety, dose and transgene expression in preparation for a Multi-dose trial (MDT) designed to assess clinical efficacy.

Methods A single nebulised and/or nasal dose of plasmid *CFTR* (pGM169)/GL67A was delivered to patients aged =16 years with a baseline FEV1 >60% predicted. Clinical and laboratory parameters were measured at intervals until day 28. A cohort of patients also underwent pre- and post-dosing (day 6 or 14) bronchoscopies for functional (airway potential difference (PD)) and molecular (QRT-PCR) evidence of vector-specific *CFTR* expression. Patients receiving a nasal dose underwent brushings for QRT-PCR and serial nasal PD measurements.

Results 35 patients received a nebulised dose of 20 ml (n=17), 10 ml (n=10) or 5 ml (n=8). A short-lived, dose-related drop in FEV1 was observed over the next 6 h (mean [SD]: 20 ml 25.7 [10.2]%; 10 ml 17.7 [9.9]%; 5 ml 13.0 [4.4]% of baseline). Subjects also experienced a systemic inflammatory response which was similarly dose-related and generally limited to the first 24–48 h post-dosing. A cohort of 6 patients (4@10 ml; 2@5 ml) received 4 g paracetamol over an 18-h period post-dosing; none of these patients developed a fever. Intriguingly, these subjects also appeared to have reduced systemic inflammatory responses. Molecular (mRNA) evidence of gene transfer was observed in some individuals from upper or lower airway brushings. On lower airway PD measurement, the majority of patients showed an increase towards non-CF values after nebulised gene therapy. 19 patients received a 2 ml nasal dose and 11 (58%) had some response in chloride secretion on nasal PD. In the two most positive individuals, responses were within the normal (non-CF) range and persisted to days 63 and 91, respectively.

Conclusions We consider the side effects after 20 ml nebulised dose excessive for repeated application. Those at 10 and 5 ml were more acceptable. Gene expression was confirmed in some patients, and restoration of CFTR function to the non-CF range has been observed out to 13 weeks following a single nasal dose. These data support progression of this agent to MDT.

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T5 THE KCa3.1 K+ CHANNEL MEDIATES WOUND HEALING IN HUMAN MYOFIBROBLASTS

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[1]K M Roach, [2]W Coward, [3]C Feghali-Bostwick, [1]S M Duffy, [1]P Bradding. [1]*University of Leicester, Leicester, UK;* [2]*University of Nottingham, Nottingham, UK;* [3]*Department of Medicine, Division of Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA*

Idiopathic pulmonary fibrosis (IPF) is a common progressive interstitial lung disease and current treatments are ineffective. The Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ modulates the activity of several structural and inflammatory cells which play important roles in model diseases characterised by tissue remodelling and fibrosis. We hypothesise that $K_{Ca}3.1$-dependent cell processes are a common denominator in IPF. We have therefore examined $K_{Ca}3.1$ expression and function in human myofibroblasts derived from patients with IPF and non-fibrotic controls (NFC). IPF tissue was obtained from diagnostic lung biopsies, and NFC tissue from healthy lung removed

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